REMARKS

Claims 1-27 are pending in this application.

The specification and claims 7-27of the instant application have been amended to comply with 37 C.F.R. §§ 1.821-1.825. Specifically, the specification has been amended by inserting sequence identifiers from the Sequence Listing filed herewith. Claims 7-27 have been amended to correct typographical errors, to remove improper multiple dependencies, to define variables and to recite sequence identifiers. Support for these amendments to the specification and claims can be found throughout the application as filed. Specific support for amended claim 10 can be found in the specification at page 7, lines 15-21. Applicants respectfully submit that no new matter has been introduced via these amendments to the application.

Applicants submit herewith a substitute Sequence Listing in compliance with 37 C.F.R. §§ 1.821-1.825. This Sequence Listing is submitted in lieu of the Sequence Listing filed August 13, 2001. Both a paper version of the Sequence Listing and a computer readable format containing the same information as the paper version of the Sequence Listing are enclosed. The substitute Sequence Listing does not include new matter. Entry is respectfully requested.

An early and favorable Action on the merits is respectfully requested. Notification to that effect is earnestly solicited. Should questions regarding

patentability arise, the Examiner is invited to telephone the undersigned to discuss the same.

Respectfully submitted,

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APPENDIX
MARK UP VERSION SHOWING CHANGES MADE

IN THE SPECIFICATION:

Paragraphs of the specification have been amended as indicated below.

Page 7, lines 12-13, insert:

In a preferred aspect of the present invention, zinc finger nucleic acid binding motifs may be represented as motifs having the following structure (SEQ ID NO.:40):

Page 8, line 20 :

Preferably, the linker is T-G-E-K or T-G-E-K-P (SEQ. ID NO.: 3).

Page 10, lines 8-12 :

Consensus zinc finger structures may be prepared by comparing the sequences of known zinc fingers, irrespective of whether their binding domain is known. Preferably, the consensus structure is selected from the group consisting of the consensus structure PYKCPECGKSFSQKSDLVKHQRTHTG (SEQ. ID NO.:1), and the consensus structure PYKCSECGKAFSQKSNLTRHQRIHTGEKP (SEQ ID NO.:2).

Page 13, lines 30-33 :

Figure 1b shows amino acid sequences of the variant α–helical regions from some zinc fingers selected by phage display using the DNA binding site gcgg**n**ggcg (SEQ. ID NO.:4) [GCGG**N**GGCG] where the central (bold) nucleotide of the middle

(underlined) triplet was either (I) 5-

Page 14, lines 6-22, insert:

Figure 1c shows a phage ELISA binding assay showing discrimination of pyrimidines by representative phage-selected zinc fingers. The matrix shows three different zinc finger phage clones (x, y and z) reacted with four different DNA binding sites present at a concentration of 3nM. Binding is represented by vertical bars which indicate the OD obtained by ELISA (Choo and Klug, (1997) Curr. Opin. Str. Biol. 7:117-125). The amino acid sequences of the variant α–helical regions from the selected zinc fingers are: REDVLIRHGK (x) (SEQ. ID NO.: 5), RADALMVHKR (y) (SEQ. ID NO.:6), and RGPDLARHGR (z) (SEQ. ID NO.:7). The DNA sequences contain the generic binding site [GCGG**N**GGCG] gcgg**n**ggcg (SEQ. ID NO.:4), where the central (bold) nucleotide was either : uracil (U), thymine (T), cytosine (C), or 5-methylcytosine (M).

Figure 2 shows the effect of cytosine methylation on DNA binding by phage-selected zinc fingers. Graphs show three different zinc finger phage binding to the DNA sequence [GCGGCGGCG] gcggcggcg (SEQ. ID NO.:4) in the presence (circle) and absence (triangle) of methylation of the central base (bold). The zinc finger clones tested contained variant α-helical regions of the middle finger as follows: (a) RADALMVHKR (SEQ. ID NO.:6), (b) RGPDLARHGR (SEQ. ID NO.:7) and (c) REDVLIRHGK (SEQ. ID NO.: 5). The respective zinc finger clones preferentially bind their cognate DNA site in the presence, absence, or regardless of cytosine methylation.

Page 17, lines 24-25, insert:

A "leader" peptide may be added to the N-terminal finger. Preferably, the leader peptide is MAEEKP (SEQ ID NO.:39).

Page 28, lines 21-22, insert:

[5'CTCCTGCAGT TGGACCTGTG CCATGGCCGG CTGGGCCGCA TAGAATGGAA CAACTAAAGC3'] 5'ctcctgcagt tggacctgtg ccatggccgg ctgggccgca tagaatggaa caactaaagc 3' (SEQ ID NO.:11).

Page 32, lines 20-27, insert:

DNAs of the form [5'-tatagtg-XXXX-GGCGtgtcacagtcagtccacacacgtc-3'] 5'-tatagtg-xxxx-ggcgtgtcacagtcagtccacacacgtc-3' (SEQ. ID NO.:12), and their complementary strands, are chemically synthesized [syntehsised] and annealed in 20mM Tris-HCl, pH 8, 100mM NaCl. The DNA sequences –XXXX-represent nucleotide sequences after methylation by M.*Hae*III (GGMC) or M.*Hha*I (GMGC). Since DNA is chemically synthesized, the DNA sites used in selections incorporate 5-meC (in appropriate positions on both strands) with 100% yield. Selections are also carried out on derivatives of these sites containing thymine rather than 5-meC in the appropriate positions (and with A rather than C on the complementary strand as appropriate).

Page 37, lines 1-10, insert:

apparent Kd of each clone for the optimally bound DNA site(s) is in the

nanomolar range, similar to that of wild-type Zif268 DNA-binding domain for its preferred target site using this assay. The K_ds obtained are shown in Table 2. Clones zfHAE(M) (Table 1 F1: SEQ ID NO.:20; F2: SEQ ID NO.:25; F3: SEQ ID NO.:30) and zfHHA(M) (Table 1 F1: SEQ ID NO.:21; F2: SEQ ID NO.:26; F3: SEQ ID NO.:31) preferentially bind their respective DNA target sites when 5-meC is incorporated into the correct nucleotide positions, and discriminated against the unmethylated DNA sites by factors of approximately 20-fold and 5-fold, respectively. The discrimination shown by zfHAE(M) in particular is good considering the simple DNA recognition mechanism of zinc fingers, and that only a single functional group per DNA molecule has been altered Clones zfHAE(Y) (Table 1 F1: SEQ ID NO.:23; F2: SEQ ID NO.:27; F3: SEQ ID NO.:32) and zfHHA(Y) (Table 1 F1: SEQ ID NO.:24; F2: SEQ ID NO.:28; F3: SEQ ID NO.:33) bind their respective target sites but do not show any preference for either the modified or unmodified forms.

Page 39, lines 28-29, insert:

The dissociation constants for the interaction seen between zfHAE(M), zfHHA(M) and zfHAE(T) (Table 1 F1: SEQ ID NO.:24; F2: SEQ ID NO.:29; F3: SEQ ID NO.:34) and 5-meC or T oligonucleotides are set forth in Table 3.

IN THE CLAIMS:

The claims have been amended as indicated below.

7. (Amended) The [A] method according to claim 5 or claim 6, wherein the modified residue is 5-meC and the unmodified residue is C.

8. (Amended) The [A] method according to claim 5 or claim 6, wherein the modified residue is U and the unmodified residue is T.

9. (Amended) The [A] method according to [any one of claims 5 to 8] claim 5 or claim 6, wherein the library is screened by phage display.

10. (Amended) The [A] method according to [any one of claims 5 to 9] claim 6, wherein each zinc finger has the primary structure of (SEQ ID NO.:40):

X^a **C** X_{2-4} **C** X_{2-3} F X^c X X X X L X X **H** X X X^b **H**- linker,

-1 1 2 3 4 5 6 7 8 9

wherein each of X, X^a, X^b and X^c is any amino acid, and

wherein X_{2-4} means either 2 or 4 amino acids are present at this position, and X_{2-3} means either 2 or 3 amino acids are present at this position.

11. (Amended) The [A] method according to claim 10, wherein X^a is $^F/_y$-X or P-$^F/_Y$-X.

12. (Amended) The [A] method according to claim 10 or claim 11, wherein X_{2-4} is selected from the group consisting of S-X, E-X, K-X, T-X, P-X and R-X.

13. (Amended) The [A] method according to [any one of claims 10 to 12]

claim 10, wherein X^b is T or I.

14. (Amended) The [A] method according to [any one of claims 10 to 13] claim 10, wherein X_{2-3} is selected from the group consisting of G-K-A, G-K-C, G-K-S, G-K-G, M-R-N and M-R.

15. (Amended) The [A] method according to [any one of claims 10 to 14] claim 10, wherein the linker is T-G-E-K or [T-G-E-K-P] the sequence set forth in SEQ ID NO.:3.

16. (Amended) The [A] method according to [any one of claims 10 to 16] claim 10, wherein position +9 is R or K.

17. (Amended) The [A] method according to [any one of claims 10 to 16] claim 10, wherein positions +1, +5 and +8 are not occupied by any [one] of [the] hydrophobic amino acids [,] F, W or Y.

18. (Amended) The [A] method according to claim 17, wherein positions +1, +5 and +8 are occupied by residues K, T and Q respectively.

19. (Amended) A method for preparing a DNA binding polypeptide of the Cys-2-His zinc finger class capable of binding to a DNA triplet in a target DNA sequence comprising 5-meC, but not to an identical triplet comprising unmethylated C comprising:

a) selecting a model zinc finger domain from the group consisting of naturally occurring zinc fingers and consensus fingers; and

b) mutating the finger by the method of any one of claims 3 to 5 [17].

20. (Amended) The [A] method according to claim 19, wherein the model zinc finger is a consensus zinc finger whose structure is selected from the group consisting of the consensus structure [PYKCPECGKSFSQKSDLVKHQRTHTG] set forth by SEQ ID NO.:1 and the consensus structure [PYKCSECGKAFSQKSNLTRHQRIHTGEKP] set forth by SEQ ID NO.:2.

21. (Amended) The [A] method according claim 19 wherein the model zinc finger domain is a naturally occurring zinc finger whose structure is selected from one finger of a protein selected from the group consisting of Zif 268 [(Elrod-Erickson et al. (1996) Structure 4:1171-1180)], GLI [(Pavletich and Pabo, (1993) Science 261:1701-1707)], Tramtrack [(Fairall et al., (1993) Nature 366:483-487),] and YY1 [(Houbaviy et al., (1996) PNAS (USA) 93:13577-13582)].

22. (Amended) The [A] method according to claim 21, wherein the model zinc finger is finger 2 of Zif 268.

23. (Amended) The [A] method according to any one of claims [3 to 22] 3, 4

or 5, wherein the binding protein comprises two or more zinc finger motifs, placed N-terminus to C-terminus.

24. (Amended) The [A] method according to claim 22, wherein the N-terminal zinc finger is preceded by a leader peptide having the sequence of [MAEEKP] SEQ ID NO.:39.

25. (Amended) The [A] method according to claim 23 [or claim 24], wherein the DNA binding protein is constructed by recombinant DNA technology, the method comprising the steps of:

a) preparing a DNA coding sequence encoding two or more zinc finger binding motifs preparable according to claim 23 [claims 23 or 24], placed N-terminus to C-terminus;

b) inserting the DNA sequence into a suitable expression vector; and

c) expressing the DNA sequence in a host organism in order to obtain the DNA binding protein.

26. (Amended) The [A] method according to any one of [claims 3 to 25] claims 3, 4 or 5 further comprising the [additional] steps of subjecting the DNA binding protein to one or more rounds of randomization [randomisation] and selection in order to improve the characteristics thereof.

27. (Amended) A zinc finger polypeptide which binds to a target DNA sequence containing a modified base but does not bind to an identical

sequence containing the equivalent unmodified base, preparable by a method according to any one of claims [3 to 26] 3, 4 or 5.